THE DIFFERENTIATION OF GEOGRAPHICAL GROUPS IN LYMNAEA PALUSTRIS

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Introduction

This paper is a first report on a part of the experimental work on individual and group differences in *Lymnaea palustris,* reared under standardized laboratory conditions during the years 1937 to 1940. The actual experiments were conducted jointly by the two authors. The analysis of the data and their presentation in this paper are primarily the work of the first author, who wishes at this point to express full appreciation of the advice and assistance given by Professor Frederick E. Croxton and Mr. Richard H. Brown in connection with the statistical analysis.

The investigation began with a study of *Lymnaea rubella,* indigenous to the Hawaiian Islands, and was later extended to include the local species, *Lymnaea palustris,* for comparison. Two independent collections of *Palustris* were made, one by the second author from a small stream not far from Croton, New York, and the other by the first author from a mill-pond in Newtown, Connecticut. These groups are herein designated as *Lymnaea palustris* Newtown and *Lymnaea palustris* Croton. It soon became clear that the Newtown snails, though morphologically indistinguishable, differed widely from the Croton snails in their physiological processes of growth, fertility, and longevity. The problem was to determine whether these physiological differences were of sufficient magnitude to be significant characteristics of two distinct geographical groups or races, or whether they were to be regarded as the expression of individual variation within one group or population. The problem is of fundamental importance for the analysis of evolution.

An extensive review of the literature is unnecessary here. The problems presented by geographical races are fully discussed by Robson and Richards (1936), Dobzhansky (1937), and by the collaborators of the recent volume " The New Systematics " edited by Huxley (1940); noteworthy chapters in the last-named are those by Huxley (1940), Muller (1940), and Diver (1940). Diver's (1939) general discussion of racial variation must also be mentioned.

For the most part, morphological characters of diverse local groups have been investigated. As representative studies, we may cite those of Rensch (1929) on birds, Gulick (1905) on Achatinellidae, Bartsch (1920) on *Cerions,* Fischer-Piette (1938) on *Patella,* Crampton (1916, 1925, 1932) on *Partula;* Goldschmidt (1934) on *Lymantria,* and Mozley (1935) on *Lymnaea.*

Dobzhansky (1935) has demonstrated that in *Drosophila pseudoobscura* there are two races which are morphologically indistinguishable but physiologically unlike. Working with the same material, Shapiro (1932) and Poulson (1934) have likewise shown that physiological differences between two races exist, while Lancefield's investigation (1929), confirmed by Dobzhansky and Boche (1933), proved that the two races can be distinguished also by the differing forms of the Y chromosome. Recently, Baily (1939) has published an important study on "Physiological Group Differentiation in *Lymnaea columella.*"

Our methods of culture of *palustris,* as well as the adopted treatment on the data, differ from those employed by Baily in his work on *columella.* No prior physiological studies on *palustris* are known to us. This species of *Lymnaea* is very different from *columella* and also from *rubella* in its physiological processes of growth and reproduction. It is intermediate between them in the length of its life cycle.

The animals of our experiments were kept in a greenhouse during the winter months in quart jars filled with water to approximately the 500-cc. level. Efforts were made to keep the temperature between 55° and 75° F.; the optimum seemed to be from 60° to 70°. When the temperature grew too warm, toward the end of May, the snails were transferred to cooler laboratories, there to remain until the end of September, when they were returned to the roof greenhouse.

Spring water was used consistently because the animals were adversely affected by the city water. Each jar contained a spray of Cabomba, to provide a substratum for the deposition of eggs, as well as a small amount of finely-sifted earth. The standard food was lettuce of the "Boston" variety, and some of this was always available in the jars.

The individual masses of eggs were removed from the jars soon after they were laid, and were then kept in glass cups of 50-cc. capacity until the young began to hatch. Each cup with its contents was then placed in a standard quart jar where the growing animals passed their early lives in company. Obviously all of the products of a single mass of eggs were kept under identical conditions. When the young snails reached the age of eighty days, they were measured and isolated in the standard quart jars. Thereafter they were re-measured at eighty-day intervals, and full records of their fertility were secured until they died.

While some of the original wild individuals may have mated before they were collected, the isolated members of the later generations could not have done so. Therefore all of the eggs deposited by the snails of the pedigreed generations were the products of self-fertilization on the part of the animals. As two polar bodies are formed, the possibility of parthenogenesis is excluded. The statistical methods employed in the present study are those which have been developed by Fisher (1938), Mahalanobis (1932), and Snedecor (1934, 1937) for the analysis of variance. When two groups are compared on the basis of measurable characters, the difference between the means may be estimated as significant of a real distinction by the use of the F test. Technically, F is the ratio of the variance between the means to the variance within the separate groups. If the variance between the means is so great as compared with the variance within the separate groups that the variance between the means cannot be regarded as happening by chance, then the hypothesis that the groups have been drawn from the same population must be rejected. It follows then that the two groups show a significant difference, i.e. a difference which is not attributable to chance variation.

In our tables N is the number of individuals in the sample; $\overline{X}$ is the arithmetical mean, and r is the conventional coefficient of correlation; while F is a critical value which, when taken in conjunction with the proper degrees of freedom, can be denoted as significant or not significant by reference to an F table such as that available in "Applied General Statistics" (pp. 878–879) by Croxton and Cowden. The degrees of freedom involved are: between the means, $n_1 = 1$; and within the samples, $n_2 = N_1 - 1 + N_2 - 1$, where $N_1 =$ the number of items in one sample, and $N_2 =$ the number of items in the other sample.

Fertility

The term fertility as used in this report refers to the egg-laying activity or productivity of the snails. It has no reference to the viability of the eggs or of the later embryos and growing young.

The original *Lymnaea palustris* constituting the first, or parent generation was collected in October, 1937. It comprised snails that had hatched during the previous spring or early summer, as judged by the observations on the growth and life cycles of later generations. The animals were large enough and old enough to lay soon after they were brought into the laboratory. Only15 of the 34 Newtown snails and 14 of the 45 Croton snails produced any eggs, whatsoever. In nature these animals are relatively inactive in winter; we found that no eggs were laid by individuals kept at low temperatures in a cold room.

As shown in Fig. 1, both groups of snails began to lay abundantly during March, and reached the peak of egg production in April. Egg-laying by the Newtown snails then fell off rapidly to a low level in August, after which it was sporadic until December, when the last Newtown snail died at an age of approximately a year and a half. In

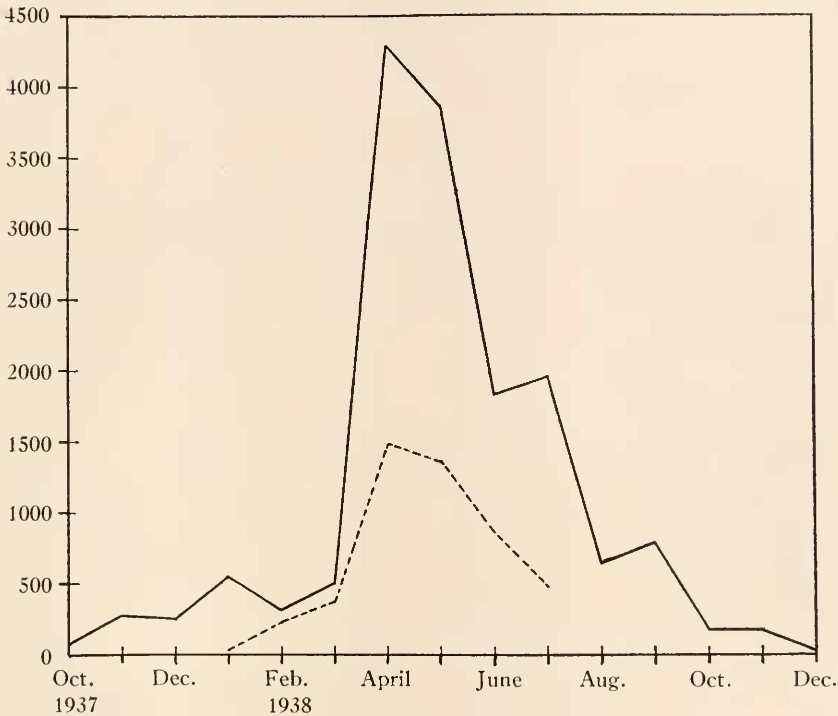

FIG. 1. Egg-laying cycles of the Newtown snails (solid line) and of the Croton snails (broken line) of the parent generation. Y axis—number of eggs produced: 500, 1,000, 1,500, 2,000, 2,500, 3,000, 3,500, 4,000, 4,500. X axis—calendar months: October, 1937 to December, 1938.

contrast, the Croton snails stopped laying quite abruptly in July and they died soon afterward, apparently having reached the end of their normal life cycle, for members of the succeeding generations of this group seldom lived longer than 240 to 320 days. Possibly the heat waves of July hastened their deaths, for it was found later that the Croton snails did not survive extreme hot weather as well as did the Newtown animals.

In comparing the fertilities of the Newtown and Croton groups, several characteristics of reproduction and of the reproductive cycle were taken into account; namely, the number of egg masses laid, the total number of capsules containing fertile eggs, the number of empty capsules laid, the age of the animal at the onset of fertility, and the length of the fertile period. Table I gives a comparison of the Newtown group with the Croton group in the first, or parent, generation. Each group comprised 15 laying individuals.

The F tests show that the Newtown animals were the more productive. They also had a longer fertile period and they laid fewer empty capsules. Further, the Newtown group laid more egg masses than the Croton group, as the means show, but the difference in this respect is not mathematically significant. Colton's statement that the laying of empty capsules comes at the end of the reproductive cycle in *columella,* as a sign

TABLE I

Comparative fertility of the Newtown and Croton snails of the parent generation

Character	N	$\bar{X}$	F	Significance
Number of egg masses				
Newtown	15	54.4		
Croton	15	32.0		
			3.332	none
Number of eggs laid				
Newtown	15	1049.80		
Croton	15	320.00		
			9.334	positive
Number of empty capsules				
Newtown	15	5.33		
Croton	15	22.73		
			6.105	positive
Length of fertile period in days				
Newtown	15	122.27		
Croton	15	62.27		
			5.486	positive

of decadence, is borne out by our experience with that species, but in *palustris* this does not seem to be equally true.

Egg production in both groups was ascertained for successive 10-day periods for each snail. In each series the peak of production came in the first or the second 10-day period after the onset of fertility. During the first 20 days of laying, the Croton snails produced 968 eggs in 71 egg masses; the Newtown snails laid 2506 eggs in 91 egg masses. After the peak, production fell off gradually until the 151–160-day period for the Croton group and the 281–290-day period for the Newtown series, and soon afterwards the last survivors died.

The second generation, so-called, comprises the offspring of the original or parent animals. The series described by the figures of Table

II were selected from families whose reproductive cycles were approximately synchronized as to the season of the year. In addition, other calculations were made using random samples of all of the families of this generation and the results are given in Table III. Clearly the group differences that appeared in the first generation were consistently repeated in the second generation, as regards the total number of eggs, the number of empty capsules, and the length of the fertile period. In addition, there was a marked difference between the two series in the age at onset of fertility; here there is no comparison to be drawn with the parent generation, since the ages of the latter were only approximately known.

TABLE II

Comparative fertility of the Newtown and Croton snails of the second generation

Character	N	$\bar{X}$	F	Significance
Number of egg masses				
Newtown	24	49.96		
Croton	24	19.25		
			12.265	positive
Number of eggs laid				
Newtown	24	786.33		
Croton	24	132.50		
			11.706	positive
Number of empty capsules				
Newtown	24	8.25		
Croton	24	34.46		
			8.835	positive
Age at onset of fertility in days				
Newtown	19	180.63		
Croton	47	97.38		
			122.621	positive
Length of fertile period in days				
Newtown	24	98.33		
Croton	24	40.79		
			13.430	positive

The possibility of a seasonal variable in the fertility of *palustris* must be taken into account. As already noted (Fig. 1), the members of the parent generation laid few eggs during the autumn and winter and it was not until spring that they began to produce abundantly. Because the reproductive cycle of the Newtown snails differed so much from that of the Croton animals in succeeding generations, it was not possible to demonstrate a seasonal variable in their fertilities. The families compared in Table II came from eggs laid at about the same time of the year, but the Croton snails generally began to lay at earlier ages than the

Newtown snails, and they had a shorter laying span, thus making the exact synchronization of their laying records impossible. The longest laying period of the Croton group was 90 days while that of the Newtown snails was 240 days. Both groups reached the peak of production during the first two 10-day periods of their laying cycles, as in the parent generation. The Croton group actually produced more eggs during the first 20 days, but they ceased to lay much earlier than did the Newtown animals, whose total productivity was much greater in the end.

From our observations, it seems that under laboratory conditions the seasonal rhythm of laying tends to disappear, and that the onset of fertility occurs when the snails reach a certain size, regardless of the time of the year. The significant point is that the characteristic differences between the Newtown and the Croton groups of the parent generation, as regards the features of the productive cycle, were repeated in the second as well as in the later generations reared in the laboratory.

TABLE III

Comparative fertility of random samples of all Newtown and Croton snails of the second generation

Character	N	$\bar{X}$	F	Significance
Number of egg masses				
Newtown	80	39.71		
Croton	63	17.22		
			24.731	positive
Number of eggs laid				
Newtown	80	506.37		
Croton	63	139.36		
			16.192	positive
Number of empty capsules				
Newtown	80	7.76		
Croton	63	24.41		
			23.151	positive

It was impossible to procure full fertility records for all of the families of the third generation, owing to the abundance of the material. The comparison here made employs the two most productive and long-lived families of the contrasted groups (Table IV). The results of the F tests on various aspects of fertility show that the Newtown snails were again more productive than the Croton snails. While the differences are not positively significant, the animals of the former group laid more egg masses throughout a longer period of time, and their ages at the onset of fertility were greater.

Passing to the fourth generation, the Newtown group comprised the relatively large number of 278 snails; the Croton group was made up of 132 individuals belonging to five families, of which only three lived long enough to provide data for complete life cycles. Representative Newtown and Croton families are compared in Table V. Unfortunately, the number of satisfactory Croton layers is small; in fact, the Croton animals proved to be so far inferior to the Newtown snails in their fertility that their breeding had to be discontinued after this generation. The Newtown series, on the other hand, have continued to flourish. They were so weeded out that the stock of later generations has been made up of the descendants of only one of the original snails.

TABLE IV

Comparative fertility of the Newtown and Croton snails of the third generation

Character	N	$\overline{X}$	F	Significance
Number of egg masses				
Newtown	21	24.67		
Croton	12	18.17		
			1.697	none
Number of eggs laid				
Newtown	21	402.81		
Croton	12	238.83		
			4.677	positive
Number of empty capsules				
Newtown	21	2.71		
Croton	12	16.00		
			8.472	positive
Age at onset of fertility in days				
Newtown	21	133.51		
Croton	12	110.25		
			3.218	none
Length of fertile period in days				
Newtown	21	68.30		
Croton	12	52.33		
			1.328	none

The data of Table V are self-sufficient. In this generation it is again clear that the Newtown snails differed from the Croton snails in their actual productivity, and in the same ways as in the earlier series. As the physiological differences in question are exhibited by the two contrasted series throughout four generations, there can be little doubt that the Newtown and Croton groups constitute two geographical races which are presumably unlike in some particulars of their genetic constitutions.

In concluding this section, a partial record is given of the correlations displayed by the several features of fertility with one another and

TABLE V

Comparative fertility of the Newtown and Croton snails of the fourth generation

Character	N	$\overline{X}$	F	Significance
Number of egg masses				
Newtown	27	56.15		
Croton	7	6.71		
			19.615	positive
Number of eggs laid				
Newtown	26	614.46		
Croton	7	99.43		
			16.042	positive
Number of empty capsules				
Newtown	26	4.61		
Croton	7	9.86		
			3.436	none
Age at onset of fertility in days				
Newtown	26	180.77		
Croton	7	120.43		
			24.471	positive
Length of fertile period in days				
Newtown	26	123.96		
Croton	7	12.28		
			22.954	positive

with additional characters. The items of Table VI are representative of numerous calculations of data relating to many series.

As would be expected, productivity as indicated in terms of the number of egg masses and of the number of fertile eggs is positively correlated with the length of the fertile period. The total number of eggs laid and the length of the fertile period are correlated with age at death to significant degrees. It is otherwise with the age at onset of fertility, for this feature does not show a definite correlation either with total productivity or with age at death.

TABLE VI

Correlations in three Newtown families of the second generation taken as random samples

Characters	N	r	F	Significance
Number of egg masses and length of fertile period	24	.901	94.897	positive
Number of eggs and length of fertile period	24	.743	27.107	positive
Number of eggs and age at death	40	.532	15.016	positive
Length of fertile period and age at death	40	.806	70.447	positive
Age at onset of fertility and total productivity	40	.258	2.719	none
Age at onset of fertility and age at death	40	.177	1.229	none

Growth

The main purpose of this section is to compare the Newtown and Croton groups of *palustris* on the basis of the mode of growth and of size at certain stages in the life cycles of the snails. It will appear that differences in these characteristics are quite as definite as in the physiological attributes discussed under the head of fertility.

It will be recalled that the young snails hatched from a single mass of eggs were reared in one and the same nursery jar until they were 80 days old, at which time they were measured and isolated in individual

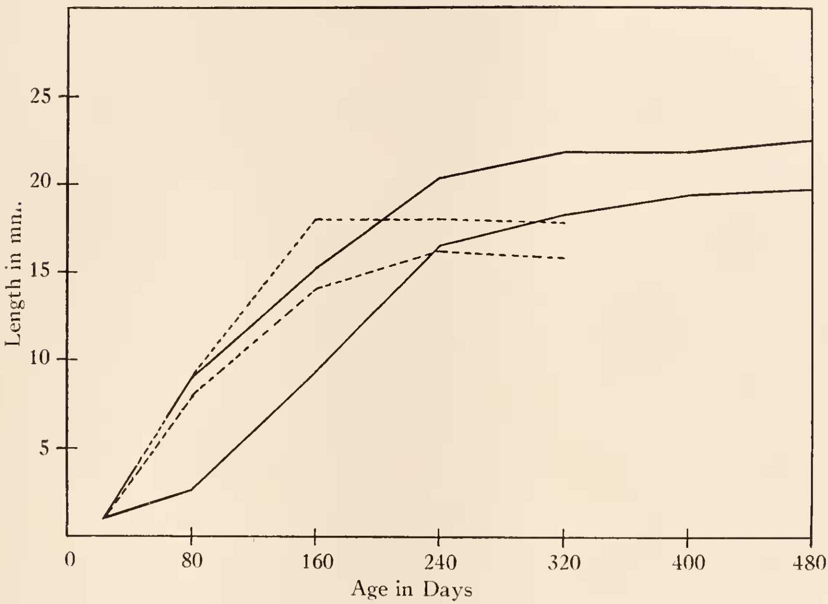

Fig. 2. Growth curves of the fastest-growing and the slowest-growing snails of a typical Newtown family (solid line) and of a typical Croton family (broken line). Y axis—length in mm.: 5, 10, 15, 20, 25. X axis—age in days: 80, 160, 240, 320, 400, 480.

quart jars, thereafter to be measured at 80-day intervals. The total length of the shell was taken as the index of size; measurements of width were also made, but they have not been employed in the present study. In Baily's work on *columella* (1939), the length of the aperture was used as an index of size. Baily measured his animals every five days and thus obtained enough data to plot a logistic curve of growth for each of his two comparable groups. Merrell (1931) investigated the growth of rabbits and plotted a similar logistic curve for each individual and for the whole series of observed animals. Our own measurements are relatively few, but they describe the animals in definite terms at actual successive age-periods in their several cycles. Whatever their limitations, they provide a concrete basis for the determination of group averages and for an estimate of the significance of group differences by the use of the F test. The results are given in Fig. 2 and in Tables VIII to XI.

The length of approximately 1 mm. at about 20 days is taken as the starting point of the curves of growth, even though variations are found at this time and even earlier, just as they are throughout the entire life cycle. During the first hour after a mass of eggs is deposited, water is absorbed by the gelatinous matrix, by the individual egg-capsules, and by the actual eggs themselves, with some degree of enlargement as a consequence. The eggs remain the same in size after the first hour until they begin to divide, about three hours after deposition. The diameter of the eggs of one representative series of *palustris* ranged from 0.108 mm. to 0.126 mm., with an average of 0.115 mm. and a coefficient of variation of 5.36. The early embryos vary considerably even before they reach the time of hatching, and there are also considerable differences between broods as regards the amplitude of variation before emergence. In one Newtown series numbering 30, measured at 13 days, the length ranged from 0.882 mm. to 1.008 mm., the average was 0.952 mm., and the coefficient of variation was 3.69. In another Newtown series of 36, measured at 14 days, the length varied from 0.720 mm. to 1.134 mm., the average was 0.948 mm., and the coefficient of variation was 12.26. It is probable that the larger eggs produce relatively larger embryos. Künkel (1916) has proved this to be the case in *Arion* and in other land pulmonates. But it does not *necessarily* follow that the individuals which are larger at the time of hatching remain comparatively larger throughout their later lives.

The data of size as indicated by length are given in Table VII for the successive ages of representative broods of *palustris;* the LP1 and LP15 families belonged to the Newtown group while LP63 was a Croton snail. The figures are self-explanatory. They show how the illustrative families differ at the several age-intervals, and how their averages tend to converge in the latter portions of the life cycle. As a matter of detail, in some instances the average size is sometimes less at an older age than it was at an earlier time owing to the death of relatively larger individuals; a case in point is LP15-A at 400 days. In general, absolute variation as indicated by its coefficient is relatively greatest at the 80-day stage and it diminishes as the animals grow older, although there are notable exceptions.

The figures of Table VII and the curves of Fig. 2 show that the contrasted Newtown and Croton groups differ in their modes of growth. The Newtown animals generally increase in size at a slower rate at the outset, but they become comparatively larger at later times and they attain their maximum dimensions at about 320 days. The Croton snails have a shorter life-span as a rule, and they reach their lesser limits of growth by about 240 days—a point that is brought out more definitely in the subsequent section on longevity. The few individuals in every

TABLE VII

Data of size and growth of representative families of the second generation

Age	Family	No.	Arithmetical mean	Standard deviation	Coefficient of variation
days			*mm.*		
80	LP1–B	38	7.657	1.072	14.00
	LP15–A	11	9.463	2.866	30.29
	LP15–S	23	7.000	1.206	17.23
	LP63–K	74	8.605	2.257	26.23
160	LP1–B	35	10.011	1.463	14.62
	LP15–A	9	13.922	1.127	8.09
	LP15–S	22	21.368	1.317	6.16
	LP63–K	47	17.772	2.071	11.66
240	LP1–B	9	16.622	2.960	17.81
	LP15–A	5	21.000	3.130	14.90
	LP15–S	12	24.525	1.879	7.66
	LP63–K	20	18.525	1.394	7.52
320	LP1–B	5	21.760	2.365	10.87
	LP15–A	3	22.867	3.412	14.92
	LP15–S	7	24.415	1.397	5.72
	LP63–K	4	16.650	0.870	5.22
400	LP1–B	5	22.700	1.227	5.40
	LP15–A	2	21.400	2.545	11.90
	LP15–S	1	24.000	.....	.....
	LP63–K	0	......	.....	.....

family that live beyond the times specified may not increase in size, but if they do, the increments of growth are small.

The contrasted distinctions of the Newtown and Croton groups are consistently the same in all of the material of the second generation. The series described in Table VIII differ somewhat from those of Table VII. The Croton group as a whole is definitely larger in size at 80 days while the members of the Newtown group are the larger at 160 days and 240 days with equal definiteness. An additional calculation

TABLE VIII

Comparative Sizes of the Newtown and Croton snails of the second generation

	N	$\overline{X}$	F	Significance
		mm.		
Size at 80 days				
Newtown...........	38	7.66		
Croton.............	74	8.60		
			5.981	positive
Size at 160 days				
Newtown...........	58	19.97		
Croton.............	47	17.77		
			11.570	positive
Size at 240 days				
Newtown...........	32	21.37		
Croton.............	23	18.41		
			13.865	positive

of 80-day sizes was made for the entire populations belonging to this generation with corroborative results; specifically, the mean size for the Newtown group was 7.150 mm. and the mean of the Croton group was 8.845, with a value for F of 15.662 that is certainly positive in significance.

When the third and fourth generations were analyzed (Table IX and Table X), the Newtown and Croton groups showed the same distinctions so far as the conditions at 80 days are concerned. At the 160-day period, the Croton snails were again smaller collectively in the third generation, but they were relatively larger in the fourth generation, although in neither case were the differences significant. In two respects the relations at 240 days were not the same as they were in the second

TABLE IX

Comparative sizes of the Newton and Croton snails of the third generation

	N	$\overline{X}$	F	Significance
		mm.		
Size at 80 days				
Newtown..........	261	6.67		
Croton............	19	9.94		
			20.461	positive
Size at 160 days				
Newtown..........	251	16.73		
Croton............	18	15.91		
			1.284	none
Size at 240 days				
Newtown..........	222	18.07		
Croton............	9	16.58		
			3.691	none

TABLE X

Comparative sizes of the Newtown and Croton snails of the fourth generation

	N	$\overline{X}$	F	Significance
		mm.		
Size at 80 days				
Newtown..........	132	5.65		
Croton............	86	7.69		
			36.645	positive
Size at 160 days				
Newtown..........	44	14.69		
Croton............	43	14.98		
			0.210	none
Size at 240 days				
Newtown..........	38	17.13		
Croton............	9	19.27		
			9.139	positive

generation. In the first place, the Croton snails did not differ significantly from the Newtown group although they were collectively smaller as before. In the second place, the Croton snails proved to be definitely *larger* at 240 days than the Newtown animals of the same age.

There is no question that the Newtown and Croton groups differ distinctly in mode of growth and in relative size during the earlier months of their life cycles, in all of the pedigreed generations. The circumstances during the later months of life are not so consistent. The reasons why the later generations fail to agree with the second generation are unknown. It may be that unconscious and unavoidable selection is responsible in part, and it may also be that the conditions of laboratory culture exert a modifying influence on the rates of growth in the course of time. However, the Newtown and Croton groups do show differences in certain physiological qualities of growth during their earlier lives, and these differences indicate that the two groups are justly to be regarded as distinguishable geographical races.

TABLE XI

Correlations of size and growth in Newtown snails of the third generation

Characters	N	r	F	Significance
Size at 80 days and size at 320 days..............................	87	+.582	43.535	positive
Size at 80 days and increment of size from 80 to 160 days........	87	−.719	91.075	positive
Size at 320 days and increment of size from 80 to 160 days........	87	+.216	4.160	positive

Certain correlations of size and mode of growth were determined for some of the series, and the results are given in Table XI. The figures relate to random samples of the Newtown group of the third generation. Size at 80 days and size at 320 days are positively and definitely correlated. In general, the individuals which grew more slowly from the time of hatching to the age of 80 days were the smaller at the last. In other words, the animals tend to retain their respective positions in the graduated scale, from an early age to the virtual end of growth.

The increment of growth from 80 days to 160 days is *negatively* and significantly correlated with size at 80 days. It is clear that the larger snails of the 80-day array grew more slowly during the following interval in question in comparison with the smaller ones, even though the latter generally failed to overtake the former, as the previous correlation has shown. Obviously the form of the curve of growth varies. In some animals it rises rapidly and more precociously to the 80-day stage and thereafter the rate of increase gradually diminishes. In other instances the curve rises more slowly at first, and the transition to a period of more rapid growth falls beyond the 80-day stage. The positive correlation between size at 320 days and the growth increment from 80 days to 160 days is an incidental feature of some interest.

The average age at the onset of fertility is definitely correlated with size at 80 days, with a negative sign. The snails which were relatively larger at 80 days were generally the first to produce eggs. In a Newtown random sample of 12, r was —.766 and F was the significant figure of 14.238; in a comparable random sample of 63 Croton snails r was —.737 and the value of F, namely, 68.242, was also a significant figure. The two groups show exactly the same correlations even though they differ markedly in their mean ages at the onset of fertility as shown in the previous section.

Longevity

Discussion of the interesting problem of longevity is here confined to the contrast between the Newtown and Croton groups of *palustris*. No data are available for the members of the parent generation because their exact ages when they were brought into the laboratory were unknown; but the following generations have provided abundant information on the length of life of the snails from the date when the eggs were laid to the time of death. No records were obtained for the animals that died before the age of 80 days, the time of isolation; therefore our calculations are based on random samples of those snails which were alive at the age specified.

From the figures of Table XII it is obvious that the Newtown snails of the 80-day population lived longer than the comparable series of Croton snails. The differences between the two groups are consistent and significant throughout all three generations, and they provide additional evidence that the Newtown and Croton groups constitute different geographical races. A minor point of interest is that erosion of

TABLE XII

Comparative length of life of the Newtown and Croton snails

Generation	N	$\bar{X}$	F	Significance
		days		
Second				
Newtown	69	245.13		
Croton	71	194.87		
			12.155	positive
Third				
Newtown	23	330.82		
Croton	22	216.77		
			39.567	positive
Fourth				
Newtown	31	332.39		
Croton	23	206.83		
			25.962	positive

the shell—an indication of senescence—often occurred in the Croton snails at or before 240 days, while in the Newtown snails it was seldom observed until after 320 days.

A positive and significant correlation between size at 80 days and age at death is revealed by the records. In a random sample of Newtown snails numbering 53, r was $+ .271$ and the value F was 4.200. In general, the snails which grew more rapidly before the 80-day interval lived longer than those with a slower rate of growth in the initial stages. There is no definite correlation between size at 320 days and age of death; in a random sample of 53 individuals, the r was $+ .185$ and F was 2.367, which is too small to be significant.

Sterility

The occurrence in *palustris* of numerous individuals that did not reproduce raises questions of considerable interest. The treatment of the problems of their sterility encounters certain difficulties, partly because the age when the contrasted fertile animals begin to produce eggs is widely variable.

In the case of the original or parent generation, 19 out of 34 Newtown snails (57.3 per cent) died without laying any eggs although they grew to full size, while 31 of the 45 Croton snails (68.9 per cent) were also sterile. Although their proportionate numbers varied from family to family, some of the members of the later generations failed to produce eggs although they lived well beyond the *average* age of onset of fertility of their respective groups. In the following discussion, however, only those individuals which lived beyond the *last* onset among their associates in the group, without laying eggs, are treated as sterile.

When the fertile and the sterile series were compared on the basis of relative size at successive age intervals (Table XIII and Table XIV), the members of the productive group were uniformly the larger at all periods of the life cycle. The Newtown and Croton series displayed no differences in these relations. But the two geographical series are unlike as regards longevity (Table XV). The general age at death of the fertile animals was definitely greater in the case of the Newtown animals; in the case of the Croton series, however, the difference between the fertile and sterile components was not statistically significant, although here again the animals which produced eggs were the larger collectively. Hence sterility is certainly associated with smaller size and shorter life in the *palustris* from the Newtown area, while it is associated with smaller size, but not with shorter life, in the Croton group.

Our animals were isolated at the age of 80 days before they reproduced and any eggs which were subsequently produced were certainly the products of self-fertilization. It is possible that some of the sterile individuals might have been fertile if they had been given an opportunity to mate, but there is no way to determine whether this might have been the case.

Self-fertilization in itself does not seem to have a detrimental effect upon the viability of the young or upon the productivity of fertile individuals in *palustris;* in fact, members of the fifth, sixth and seventh generations were even more prolific than those of the second generation. Crabb (1927) states that individuals of *Lymnaea stagnalis appressa* " reared in strict isolation reproduce as abundantly as do those in mass cultures." In the work of Colton and Pennypacker (1934) on *columella,*

TABLE XIII

Comparison of size of the fertile and sterile Newtown snails of the second generation

	N	$\overline{X}$	F	Significance
		mm.		
Size at 80 days				
fertile	56	6.90		
sterile	47	5.19		
			13.839	positive
Size at 160 days				
fertile	56	17.73		
sterile	37	16.10		
			8.640	positive
Size at 240 days				
fertile	173	18.53		
sterile	28	15.21		
			17.554	positive

93 consecutive generations were reared between 1911 and 1934 with self-fertilization throughout this period of years, and at the end the animals were hardy and prolific. Incidentally, sterile individuals are far more infrequent in *columella* than they are in *palustris*. Boycott, Diver, Garstang and Turner (1930) found that in *Lymnaea peregra* the animals would mate and cross-fertilize if they were given an opportunity to do so, but if they were reared in isolation they would produce fertile eggs equally well although at somewhat older ages.

But the point of greatest interest is that in our material of *palustris* absolute sterility is associated with smaller size. This suggests that the

TABLE XIV

Comparison of size of fertile and sterile Croton snails

	N	$\overline{X}$	F	Significance
		mm.		
Size at 80 days				
fertile	63	10.09		
sterile	28	7.73		
			38.333	positive
Size at 160 days				
fertile	51	17.57		
sterile	10	14.51		
			9.045	positive
Size at 240 days				
fertile	40	18.09		
sterile	13	15.67		
			22.559	positive

failure to lay eggs is due to retarded development or to an actual constitutional incapacity and not to the lack of an opportunity to mate. It is possible that real recessive defects involving the reproductive apparatus and its functions may exist, and that inbreeding might therefore increase the relative numbers of infertile individuals.

Summary

Variation in *Lymnaea palustris* is exhibited in fertility, rate of growth, size, and longevity. Individual, family and group differences recur in successive generations in spite of the essential uniformity of the laboratory conditions of culture; hence their genetic causation seems probable.

Sample populations collected from two different localities and habitats were analyzed by the F test for significant differences in fertility, growth, and longevity. The results demonstrated the reality of definite differences in all three categories.

Table XV

Comparison of age at death of the fertile and sterile snails of the second generation

	N	$\overline{X}$	F	Significance
		days		
Newtown group				
fertile	157	272.94		
sterile	34	223.29		
			9.991	positive
Croton group				
fertile	62	209.37		
sterile	24	196.04		
			1.053	none

The study of group differences was continued throughout three consecutive offspring generations, and in each of these the differences demonstrated in the parent generation were found to be repeated. The two local populations are therefore to be regarded as different geographical races.

Significant and positive correlations were found between the total number of eggs laid and the age at death, between the number of eggs laid and the length of the fertile period, and between the length of the fertile period and age at death.

Extreme types of slow-growing and fast-growing individuals were found in every group with intermediates of great variety. When arranged in order of size at 80 days, the snails exhibited a tendency to keep their respective places throughout the rest of the life cycle, as indicated by the positive correlation between size at 80 days and size at 320 days. The rate of growth between 80 days and 160 days is inversely correlated with size at 80 days. The critical period in the growth curve occurs relatively early in the life cycle when the individual mode of growth appears to be established.

The onset of fertility is negatively correlated as to time with size at 80 days. The correlation in question is the same in the two local series, despite their demonstrated differences in size at 80 days and in age at the onset of fertility.

Some of the individuals in each population were sterile. When a study was made to ascertain whether such animals differed significantly from the fertile snails, the F test showed that they were collectively smaller than the productive individuals and that their lives were shorter.

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